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Two-Step Sequential Pretreatment for the Enhanced Enzymatic Hydrolysis of Coffee Spent Waste

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Abstract

In the present study, eight different pretreatments of varying nature (physical, chemical and physico-chemical) followed by a sequential, combinatorial pretreatment strategy was applied to spent coffee waste to attain maximum sugar yield. Pretreated samples were analysed for total reducing sugar, individual sugars and generation of inhibitory compounds such as furfural and hydroxymethyl furfural (HMF) which can hinder microbial growth and enzyme activity. Native spent coffee waste was high in hemicellulose content. Galactose was found to be the predominant sugar in spent coffee waste. Results showed that sequential pretreatment yielded 350.12mg of reducing sugar/g of substrate, which was 1.7-fold higher than in native spent coffee waste (203.4 mg/g of substrate). Furthermore, extensive delignification was achieved using sequential pretreatment strategy. XRD, FTIR, and DSC profiles of the pretreated substrates were studied to analyse the various changes incurred in sequentially pretreated spent coffee waste as opposed to native spent coffee waste.

Keywords: Lignocellulose; biomass pretreatment; spent coffee waste; sequential, combinatorial pretreatment; reducing sugar.

1 Introduction

Coffee is one of the most popular hot beverages and is the second most traded commodity in the world after petroleum. Global coffee production has increased by 17% from 2002 to 2014 due to its increased consumption. This gives rise to huge amounts of coffee processing residues leading to serious waste management issues (Campos-Vega et al., 2015). Spent coffee waste is generated as a result of production of instant coffee from roasted coffee beans. In 2008/09 the EU introduced 'The New Waste Framework Directive' as a new approach to curb food waste such as spent coffee waste and as well as new processes for reuse and recycling (Ravindran & Jaiswal, 2016a). Spent coffee waste is rich in a variety of organic compounds such as carbohydrates, lignin, fatty acids and antioxidant compounds which makes it an excellent candidate for the production of value-added substances (Scully et al., 2016; Ballesteros et al., 2014).

Commercially viable goods such as bioethanol, biodiesel, sugars, activated carbon, compost and sorbent material for metal ion removal fall into the spectrum of value added products that can be derived from spent coffee waste (Go et al., 2016; Kwon et al., 2013; Utomo & Hunter, 2006).

Spent coffee waste, being lignocellulosic in nature, is mainly rich in polysaccharides, lignin, lipids and a small fraction of proteins. The polysaccharides in spent coffee waste are composed of cellulose and hemicellulose and comprise almost all of its dry weight. Cellulose is a polymer made of repeating units of glucose, while hemicellulose is heterogeneous in nature and composed of galactose, mannose and arabinose, and are structurally arranged as arabinogalactan and galactomannan (Ballesteros et al., 2015). Although high in carbohydrate content, the extraction of monomeric sugars from spent coffee waste is a tedious task. Its ability to withstand high temperatures during roasting and extraction is indicative of its recalcitrant nature.

Recalcitrance in spent coffee waste is imparted by the orderly structure of the component

material along with the presence of lignin, a phenolic polymer. Pretreatments are essential strategies that can disrupt the structure of lignocellulose for the efficient utilisation of the inherent polysaccharides (Ravindran & Jaiswal, 2016b).

Pretreatment methods are intended to increase the efficiency of lignocellulose hydrolysis by improving accessibility towards polysaccharide fractions. These strategies can be classified into physical, chemical, biological and combinatorial based on their modes of action. An efficient pretreatment strategy is generally simple to perform and releases highly fermentable products with the minimal formation of indigestible compounds. The polysaccharide fraction is least affected so that no inhibitory compounds are formed during the process. Furthermore, the energy and cost requirements should not be so high to render the whole process economically futile.

Several studies pertaining to the pretreatment of spent coffee waste for the production of value added products have been described in literature. Rocha et al. (2014) employed ultra-sonication as a pretreatment measure to produce bioethanol and biodiesel from spent coffee waste. Dilute acid hydrolysis was used as the pretreatment strategy in a study involving the extraction of sugars and lipids from spent coffee waste (Go et al., 2016). Pretreated spent coffee waste has also been used for enzyme production. Murthy and Naidu (2012) used acid and alkali pretreated spent coffee waste for the production of xylanase employing *Pencillium sp* by implementing solid state fermentation mode. However, few studies are available that investigate the effects of pretreatment methods on the various aspects of spent coffee waste.

This study proposes a two-step pretreatment strategy to increase, reducing sugar recovery after subjecting spent coffee waste to enzymatic hydrolysis. Initially, eight different pretreatments were performed for spent coffee waste and the changes in its chemical composition, structural

conformation, crystallinity and thermal behaviour was analysed and compared. All the pretreated spent coffee waste samples were then subjected to enzymatic hydrolysis using optimised parameters. Furthermore, the incidence of inhibitory compounds after each pretreatment were also investigated. Finally, two of the most effective pretreatments determined in this investigation were combined and performed sequentially to increase the sugar recovery from the enzymatic hydrolysis of spent coffee waste.

2 Materials and methods

Spent coffee waste was generously supplied by a local coffee outlet in Dublin city. The spent coffee waste was dried at 80°C for 48h and stored at room temperature in a cool and dry place for further experiments (Kwon et al., 2013). All the chemicals such as cellulase from *Trichoderma reesei*, hemicellulase from *Aspergillus niger*, conc. H₂SO₄, alkaline potassium permanganate, ethanol, ferric chloride, conc. H₃PO₄, acetone and other chemicals required for experimentation were purchased from Sigma Aldrich, Ireland. Cellulase activity was assayed by following laboratory analytical procedures for the measurement of cellulase activity devised by National Renewable Energy Laboratory (Adney & Baker, 1996). Meanwhile hemicellulase activity was assayed followed protocols described by Rickard and Laughlin (1980). Cellulase enzyme registered an enzyme activity of 77 FPU/ml while hemicellulase showed 72 U/ml enzyme activity.

2.1 Screening of various pretreatments for spent coffee waste

2.1.1 Dilute acid hydrolysis

Hydrolysis experiments were performed in an autoclave. Spent coffee waste (1% w/v) was mixed with 10 ml of different concentrations of sulphuric acid (1%, 1.3% and 1.6%) in a 100 ml

flask. The reaction mixture was subjected to 121°C for different reaction times (10, 20 and 30 min) (Zheng et al., 2013). After pretreatment the solids were separated from liquids by centrifugation at 8000 rpm for 12 min and the supernatant was analysed for total and individual sugars and by-products such as acetic acid, furfural and hydroxymethyl furfural. The solids were dried and stored in cool and dry place until further analysis.

2.1.2 Steam explosion

The steam explosion pretreatment of coffee waste was performed as described by Huang et al. (2015) with certain modifications. Briefly, five grams of coffee waste were sprayed with de-ionized water to attain 50% moisture content (w/v) and then stored at room temperature. The samples were then loaded into a stainless steel autoclave. The temperature was raised and maintained at 121°C for 30 min after which the pressure was released by opening the pressure valve subjecting the biomass to an ‘explosion’. The steam exploded coffee waste was collected, dried and stored in sealed plastic bags for further analysis.

2.1.3 Ammonia fibre explosion

Spent coffee waste (2.5 g) was soaked in 25 ml of NH_4OH and subjected to high pressure and temperature in an autoclave. The experiment was carried out at varying temperatures (70–120 °C), water loadings (0.2–2.0 ml water/g dry biomass), and residence time (1–30 min). The treated biomass was removed from the autoclave and air-dried overnight (≈ 12 h) in a fume hood to remove residual ammonia. The treated samples were stored in sealed plastic bags in a cool and dry place (Shao et al., 2013).

2.1.4 Concentrated phosphoric acid- acetone pretreatment

Pretreatment of coffee waste was performed as described by Li et al. (2009). Five grams of coffee waste were mixed well with 50 ml of concentrated phosphoric acid (85%) in a 100 ml glass beaker. The mixture was incubated at 50 °C and 120 rpm for 1 h. After the reaction, the slurry was poured into 120 ml of pre-cold acetone and centrifuged at 8000 rpm. The supernatant was collected for analysis of inhibitory compounds. The solids were mixed with distilled water and centrifuged iteratively as a means of washing off the residual acid. The solids were washed with distilled water three times followed by a fourth wash where the pH was adjusted to 6.0 with 10 M NaOH. The solids were dried and stored for further use.

2.1.5 Pretreatment using atmospheric air pressure plasma

Five grams of spent coffee waste were placed in polyethylene terephthalate (PET) trays (0.15m × 0.15m × 0.035m) which were sealed in polymeric films of thickness 50 µm thickness with very low gas transmission rates (oxygen transmission rate of $9.49 \times 10^{-9} \text{ mol m}^{-2} \text{ s}^{-1}$ at 23 °C and 101325 Pa, Cryovac BB3050, Sealed Air, Ireland). These packages were placed in the inter electrode space of the dielectric barrier discharge set up. The coffee waste samples were subjected to non-thermal plasma in triplicates for 2 min, 4 min and 6 min at three discrete voltages of 60 kV, 70 kV and 80 kV and subsequently stored for 24 h at 10°C. The pretreated coffee waste was then stored in a cool and dry place until further analysis.

2.1.6 Pretreatment using Ferric Chloride

Pretreatment of coffee waste using ferric chloride was performed according to the procedure described by Chen et al. (2015). Spent coffee waste (1% w/v) was mixed in 50 ml of 0.1M

FeCl_3 in an Erlenmeyer flask and subjected to high pressure (15 psi) and temperature (120 °C) for 30 min in an autoclave. The mixture was immediately allowed to cool down and the solids were separated from the liquid. The solids were then washed several times with deionized water to remove any residual FeCl_3 . The Fe (III) in the liquor was precipitated by gradually neutralising the solution using 0.1M NaOH. Once the precipitate was removed the salt free liquor was subjected to HPLC analysis to identify and quantify individual sugars and hydroxyl-5-methyl furfural and furfural content.

2.1.7 Organosolv Pretreatment

Organosolv pretreatment was performed as described by Ostovareh et al. (2015) with minor modifications. The coffee waste (1% dry) was mixed in 25 ml of ethanol-water mixture (50-70% ethanol (v/v) in an Erlenmeyer flask. In all experiments, 1% of sulphuric acid (w/w) per gram substrate was added as a catalyst. The reaction temperature was set at 120 °C for 30 min. After the reaction the flasks were then placed in an ice chamber to cool the contents quickly to room temperature. The contents were filtered and washed with 250 ml of 50% ethanol mixture to extract the soluble products into the liquid phase. The pretreated substrate was separated from the liquid phase by filtration and was washed several times with distilled water until pH 7 was obtained. Ethanol was evaporated from the liquid fraction and recovered by condensation. To obtain a precipitated solid, the solid fraction, mainly contained lignin, was separated by centrifugation. The pretreated coffee waste was dried and stored for enzymatic hydrolysis and further analysis.

2.1.8 Microwave assisted alkali pretreatment

Microwave-alkali pretreatment was carried out following the procedure described by Binod et al. (2012) with minor modifications. The biomass (10% (w/v)) were loaded to 1% NaOH (w/v) solution in a stoppered flask and subjected to microwave radiation at varying power settings of 240W, 400W, 560W and 800W for different residence time varying from 30 to 120 s. After pretreatment, the biomass was thoroughly washed with distilled water till pH 6.0 and air dried. The dried solid residue was used for enzymatic hydrolysis and compositional analysis.

2.2 Sequential pretreatment of spent coffee waste

Based on the observations obtained from the extensive pretreatment study conducted using coffee waste, a two-step sequential pretreatment technique was devised to achieve enhanced lignin removal, coupled by increasing yield of reducing sugars and recovery of polysaccharide content. As a consequence, concentrated phosphoric acid acetone pretreatment was improvised to include the effects of AFEX pretreatment (Fig. 1). Spent coffee waste was mixed with concentrated phosphoric acid in the ratio of 1:10 and incubated at 50 °C for 1 h in a shaker incubator at 120 rpm. The mixture was then mixed with ice cold acetone, which resulted in a slurry. The slurry was then centrifuged at 8000 rpm for 10 min to remove the solids from the liquid. The solids were washed several times to remove any reminiscent acid and acetone and then dried overnight at 80 °C. The dried coffee waste was weighed and 2.5g of the sample was mixed with 25 ml of ammonia. The mixture was then exposed to high temperature (120 °C) and pressure (15 lbs/sq.inch) in an autoclave for 30 min. The sequentially pretreated coffee waste was then kept in a fume hood overnight to remove any residual ammonia following which it was dried and stored for further analysis.

2.3 Compositional analysis

All the pretreated coffee waste samples along with their native counterpart were subjected to compositional analysis by following two stage acid hydrolysis according to the National Renewable Energy Laboratory (NREL) protocol (Sluiter et al., 2005). In brief, the coffee waste samples were hydrolysed in 72% H_2SO_4 at 30 °C for 60 min. The mixture was then diluted to 4% by adding deionised water and autoclaved for 60 min. The solids were filtered using a filtration crucible and dried at 105 °C for 48 h to remove all the moisture content. The dried solids were then burned in blast furnace for 24 h at 595 °C to obtain acid insoluble lignin. The acid soluble lignin content in the liquid was determined using spectrophotometry at 205 nm. The reducing sugar concentration in the hydrolysate was estimated by dinitrosalicylic acid (DNS) method (Miller, 1959). The identification and quantification of monosaccharides were done on an Alliance HPLC (Waters, e2695 Separation module) using a Rezex ROA-Organic acid H+ (8%) column, (350 x 7.8 mm; Phenomenex, UK) with 5 mM H_2SO_4 as the mobile phase at 65 °C maintaining a flow rate of 0.6 ml/min (Jaiswal et al., 2012).

2.4 Enzymatic hydrolysis

The enzymatic hydrolysis of spent coffee waste was performed by employing commercially available cellulase and hemicellulase purchased from Sigma Aldrich, Ireland. The hydrolysis parameters were determined employing a central composite design in an earlier study conducted in our laboratory using STATGRAPHICS Centurion XV software (Ravindran et al., 2017). Accordingly, the maximum sugar release was obtained using 1% (w/v) of pretreated spent coffee waste in a reaction volume of 50 ml at pH 6.8, cellulase loading and hemicellulase loading of 1.5 ml and 0.37 ml respectively. The hydrolysis reaction was conducted at 50 °C for 24 h.

2.5 Individual sugar and organic acid analysis

The identification and quantification of monosaccharides and organic acids was done on an Alliance HPLC (Waters, e2695 Separation module) using a Rezex ROA-Organic acid H⁺ (8%) column, (350 x 7.8 mm; Phenomenex, UK) with 0.005 M H₂SO₄ as the mobile phase at 65°C maintaining a flow rate of 0.6 ml/min. The HPLC system was equipped with an autosampler, degasser and isocratic pump and a refractive index detector. The same type of guard column was used with the regular column and was kept outside the compartment to avoid overheating beyond the manufacturers recommended limit (60 °C). An isocratic mobile phase of 0.005M sulphuric acid was used to detect and estimate the concentration of inhibitors such as furfural and hydroxymethyl furfural in the hydrolysate.

2.6 Characterization of native and pretreated substrate

2.6.1 Scanning electron microscopy

The morphological structure of spent coffee waste and the effect of pretreatment on the same were analysed by performing Field emission-scanning electron microscopy (FE-SEM, Hitachi SU-70) (Raghavi et al., 2016). Dried samples of the untreated and pretreated coffee waste were subject to FE-SEM, operating at the electron beam energy of 0.5 keV

2.6.2 X-ray diffraction

The crystalline phases in untreated coffee waste and its pretreated counterpart were analysed using X-ray diffraction. Siemens D-500 X-ray diffractometer was used for this purpose with diffraction angles spanning from $2\theta=5^{\circ}$ - 50° using a Cu K α radiation source ($\lambda=0.154$ nm). The radiation was generated at a voltage of 40 kV and current of 30 mA (Binod et al., 2012).

2.6.3 Fourier Transform Infrared Spectroscopy analysis

FTIR spectroscopy was performed on raw and pretreated coffee waste to identify the possible structural changes as a reflection of the variations in the functional groups in the coffee waste before and after pretreatment. A Perkin Elmer Spectrum GX FT-IR (UATR) Microscope (USA) was employed for this study and the FTIR spectra for different pretreated coffee waste samples were recorded from 4000 to 400 cm^{-1} with 16 scans at a resolution of 0.3 cm^{-1} in transmission mode (Raghavi et al., 2016)

2.6.4 Thermal behaviour

The thermal behaviour of the treated and raw samples was studied and compared using differential scanning calorimetry (DSC). The equipment, Shimadzu DSC-60 was operated using TA-60WS software. For analysis, 55 mg of sample was placed in an aluminium pan and an empty pan was used as a reference. The measurements were carried out between 25°C and 500°C with a linear increase of 10°C/min (Ballesteros et al., 2015).

2.6 Statistical analysis

All the experiments performed in this study were carried out in triplicates. The significant differences between pretreatment on the composition of spent coffee waste were computed by employing analysis of variance (ANOVA) and multiple comparisons (Fischer's least significant difference test). STATGRAPHICS Centurion XV software version 15.1.02 (StatPoint Technologies Inc. Warrenton, VA, USA) to determine the significant differences. Values of $P < 0.05$ were considered as significant value.

3 Results and discussion

3.1 Compositional analysis of native spent coffee waste

The chemical composition of original spent coffee waste was determined and have been summarised in Table 1. Spent coffee waste was abundant in four types of sugars *viz.* glucose, mannose, arabinose and galactose. Xylose was absent in spent coffee waste. The presence of these sugars in high amounts indicated that the spent coffee waste is rich in galactomannan, arabinogalactan and cellulose structures. These findings are in consensus with other studies on the chemical composition of the spent coffee waste (Mussatto et al., 2011). The chemical compositional analysis of the native substrate revealed that spent coffee waste is abundant in hemicellulose content (33.5g/100g) as compared to cellulose (8.6g/100g). Lignin amounted to 19.6g/100g of spent coffee waste. Of this, only 3.7g was contributed by acid soluble lignin. Variations in spent coffee waste compositions can be a result of the coffee bean raw material, the conditions that prevailed during roasting among other factors (Jooste et al., 2013).

3.2 Influence of pretreatments on composition of spent coffee waste and reducing sugar yield

Eight pretreatments were tested at various conditions to study the effect of each strategy on the composition of spent coffee waste. The final chemical composition of the biomass post pretreatment is an important factor that governs the efficiency of enzymatic hydrolysis. For a lignocellulosic substrate to be effectively used in a bioconversion process it is necessary to relatively expose cellulose fibres along with possible removal of hemicellulose and lignin fractions. Spent coffee waste was subjected to chemical composition analysis at each stage of pretreatment. The data obtained from the analysis have been summarised in Table 1.

Different experimental settings were tested for dilute acid hydrolysis (acid concentration 1%, 1.3% and 1.6%; time 10, 20 and 30 mins). High reducing sugar release was recorded for spent coffee waste pretreated with acid for 20 min at high temperature regardless of the concentration of the acid (Fig. 2a). This may be explained by the adverse impact of longer treatment times on the polysaccharide fraction of lignocellulosic biomass. Based on the reducing sugar yield, employing an acid concentration of 1.6% for 20 min was found to be the best dilute acid hydrolysis strategy for spent coffee waste (263.68 mg/g of spent coffee waste). Compositional analysis of the biomass revealed that subjecting spent coffee waste to dilute acid hydrolysis resulted in the increase in cellulose content as well as removal of considerable fractions of hemicellulose. This was evident in the reduction in the galactan, mannan and arabinan content. However, it is worth noting that acid soluble lignin was also removed from the biomass as a result of dilute acid hydrolysis, which is characteristic to this pretreatment.

Air pressure plasma gives rise to highly reactant species such as ozone, hydroxyl ions and hydronium which react with lignocellulosic polymeric structures breaking it down into component molecules. Analysis of liquor obtained after pretreatment revealed the presence of reducing sugars, which supported this fact. Air pressure plasma pretreatments were conducted using the following settings: (voltages: 60, 70 and 80 kV; time: 2, 4 and 6 mins) Increasing the intensity of plasma resulted in the reduction of reducing sugar yield. Furthermore, pretreating spent coffee waste for longer time frames was found to be detrimental to the polysaccharide content of biomass as less reducing sugar was released following enzymatic hydrolysis. It was observed that the reducing sugar release improved by exposing spent coffee waste to high intensity plasma for short time durations. Thus, subjecting the biomass to 80 kV for 4 min was found to be most effective plasma pretreatment strategy for spent coffee waste (268.68 mg of

reducing sugar/g of spent coffee waste) (Fig. 2b). This pretreatment strategy conserved much of the hemicellulose fraction along with the removal of acid insoluble lignin.

Microwave assisted alkali pretreatment was conducted in different settings of power and time (power setting: 240W, 400W, 560W and 800W; time: 30s, 60s, 120s). Any treatment beyond 120s resulted in the burning and charring of spent coffee waste. This pretreatment was effective in the removal of acid soluble lignin from spent coffee waste. The cellulose and hemicellulose fractions remained unaffected during microwave pretreated spent coffee waste. Subjecting spent coffee waste to microwave radiation at 800W for 30 s in the presence of alkali resulted in highest reducing sugar yield (258.3 mg/g of spent coffee waste). Fig. 3 shows a comparative total reducing sugar content of spent coffee waste released after enzymatic hydrolysis of pretreated samples.

Ferric chloride pretreatment increases the ease of enzymatic hydrolysis of lignocellulosic biomass by the removal of lignin and hemicellulose content. Furthermore, it changes the chemical structure and functional groups in the substrate composition to enhance the adsorption of enzymes on the cellulose. Ferric chloride pretreatment removed considerable amounts of lignin from the spent coffee waste, while cellulose and hemicellulose fractions were left intact. A reducing sugar yield of 275.4 mg/g of pretreated spent coffee waste was obtained after enzymatic hydrolysis of ferric chloride pretreated spent coffee waste. Steam explosion results in disruption of the structure and the solubilisation of the solids in spent coffee waste. The extent of solubilisation depends on the temperature applied during the process. The steam explosion of spent coffee waste resulted in marginal removal of non-carbohydrate content in spent coffee waste. The reducing sugar pertaining to steam exploded spent coffee waste post enzymatic hydrolysis amounted to 258.9 mg/g of pretreated spent coffee waste. Steam explosion was not a

particularly effective pretreatment measure for spent coffee waste. This may be because the pretreatment was conducted in an autoclave in a comparatively mild setting (121°C and 15 psi) as compared to the common practice where biomass is treated with hot steam (180 to 240 °C) under pressure of approximately 150 to 500 psi followed by an explosive decompression of the biomass. Chianzy et al. (2015) reported that steam explosion is an effective treatment for spent coffee waste.

Organosolv pretreatment was found to be detrimental to the carbohydrate content in spent coffee waste. Cellulose fibres and varying amounts of hemicellulose along lignin leach into the organic solvent used in this pretreatment. The lignin can be precipitated by simple distillation, which also results in the revival of solvent used in the process (Zhao et al., 2009). The acid catalyst used in organosolv process is corrosive in nature and can give rise to inhibitory compounds when reacting with carbohydrate fractions in lignocellulose. There was a reduction in cellulose content (7.04g/100g of spent coffee waste) although the pretreatment removed a substantial amount of acid soluble lignin. A maximum reducing sugar of 283.12 mg/g of substrate was obtained when spent coffee waste pretreated using organosolv strategy was subjected to enzymatic hydrolysis. The hemicellulose fraction was found to be fairly conserved by using this pretreatment. The galactan fraction was found to be maximum in organosolv treated spent coffee waste.

Amongst all the pretreatments tested, concentrated acid acetone pretreatment was found to be the most effective in removing mannan and acid soluble lignin from spent coffee waste. This might be due to the presence of concentrated phosphoric acid, which has been found to disrupt hemicellulose structure leading to its removal (Siripong et al., 2016). Meanwhile, AFEX pretreatment caused biomass swelling disrupting the fibres and thus breaking down linkages between lignin and carbohydrates thereby increasing the available surface area. However, this

pretreatment did not affect the composition of spent coffee waste, except for the removal of acid insoluble lignin. Of all the pretreatments tested, AFEX pretreated spent coffee waste had the least amount of acid-insoluble lignin (AIL) (12.2 g/100g of spent coffee waste). Uppugundla et al. (2014) in a study using pretreated corn stover for bioethanol production reported the decrease of AIL from 17.2g/100g of biomass to 12.2g/100g of biomass following AFEX pretreatment

Combining the varying effects of these two pretreatments was the motivation behind the sequential pretreatment strategy. Employing concentrated acid, acetone pretreatment resulted in the removal of hemicellulose and lignin content in spent coffee waste. This was followed up with the AFEX pretreatment at low temperature which resulted in the removal of acid insoluble lignin, while preserving the cellulose content (Table 1). The sequential pretreatment was highly effective in the removal of hemicellulose fractions in spent coffee waste. The galactan content which corresponds to the predominant galactose sugar in spent coffee waste was considerably reduced. Furthermore, by using sequential pretreatment a 1.7-fold (350.12 mg/g of spent coffee waste) increase was observed in the reducing sugar yield as compared to native spent coffee waste (203.4 mg/g of substrate). However, only 1.7-fold increment in reducing sugar yield may be due to the fact that coffee waste predominantly possesses less polysaccharide fraction as compared to many lignocellulosic wastes. Other factors such as the presence of lipids in the cell wall may also contributed towards hindering of efficient hydrolysis.

Analysis of variance and Fischer's Least Significant Difference (LSD) were performed to determine whether the changes in chemical composition of spent coffee waste brought about by pretreatments were significantly different to each other. ANOVA revealed that all the pretreatments were significantly different ($P < 0.05$). Table 1 provides a comprehensive idea about how each pretreatment was significantly different ($P < 0.05$) from each other in terms of different

components. Most of the pretreatments were found to be significantly different from each other. However, in certain instances no significant difference was found between two pretreatments with respect to individual components in spent coffee waste. For example, the organosolv pretreatment and steam explosion were statistically similar as far as cellulose content was concerned. The same was observed between ferric chloride pretreatment and microwave pretreatment. The changes brought about by plasma pretreatment on spent coffee waste was found to be insignificant with respect to arabinan content and acid soluble lignin content when compared to native spent coffee waste. With respect to the hemicellulose components most pretreatments were significantly different from each other. However, the sequential pretreatment did not feature any significant difference in galactan and arabinan content compared to conc. acid acetone pretreatment and AFEX pretreatment. Furthermore, the sequential pretreatment was found to be statistically similar to conc. phosphoric acetone treatment and dil. acid treatment with respect to the acid soluble lignin content. AFEX pretreatment was also unsuccessful in bringing about significant decrease in the acid soluble lignin content. Overall, the statistical analysis suggested that the sequential pretreatment was successful in combining effects of both conc. acid acetone and AFEX pretreatments.

3.3 Individual sugar and organic acid analysis

HPLC analysis of the hydrolysate obtained after enzymatic hydrolysis of each pretreated spent coffee waste identified four monosaccharides *viz.* glucose, mannose, galactose and arabinose. Kwon et al. (2013) had reported the presence of these four sugars in a study involving the use of spent coffee waste for the co-production of bioethanol and biodiesel which validated the findings of the present study. Galactose was found to be the major sugar in the hydrolysate while xylose was not observed in the spent coffee waste, which could be understood by the fact that spent

coffee waste is high in hemicellulose content. Ballesteros et al. (2015) had reported similar findings in their study on the component of sugars found in spent coffee waste. The liquor obtained after pretreatment was analysed for the presence of any individual sugars, organic acids and inhibitory compounds. All pretreatments released small but insignificant amounts of galactose and arabinose in the liquor. Interestingly, a considerable amount of furfural (12.42g/100g of SCW) was detected in the liquor obtained after organosolv pretreatment. The formation of furfural may be assisted by the presence of acid as catalysts coupled with high temperature in the organosolv process, which leads to the formation of hydroxyl methylfurfural as a result of degradation of monosaccharides.

3.4 SEM, XRD and FTIR profiles of untreated and pretreated spent coffee waste

SEM was employed to study the structural changes in spent coffee waste brought about by the sequential pretreatment process. Spent coffee waste is different from other lignocellulosic substrates in terms of particle shape, size and nature. It is highly porous, non-fibrous and exhibits a sheet like structure (Ballesteros et al., 2014). The main effects that can be observed in the SEM images of spent coffee waste is the reduction in size. Smaller particles were easily identified in the pretreated spent coffee waste SEM image. This can be attributed to the efficiency of the pretreatment in the removal of hemicellulose structures which otherwise would have physically hindered the accessibility of the enzyme to cellulose. Furthermore, the hemicellulase fraction would have adsorbed the hydrolytic enzymes, resulting in low enzyme concentration. Extensive disintegration of cell structure of the biomass was achieved by the combination of two strategies resulted in a substantial increase in external surface area. All these factors contributed to a better saccharification of pretreated spent coffee waste.

The effects of the various pretreatments applied on spent coffee waste can be analysed by changes in functional group pertaining to the lignocellulosic biomass. These changes were analysed by FTIR. The band at 897 cm^{-1} represents changes in the β -glycosidic linkages between cellulose and hemicellulose and any changes in this band represents the intermolecular degradation in the hemicellulose structure which occurs due to its removal. Decrease in absorbance at 1035 cm^{-1} is indicative of the stretching of bonds C-O, C=C and C-C-O that exist between the polysaccharide and lignin fraction. The bands in the range 1000 cm^{-1} to 1200 cm^{-1} indicate cellulose regions. The decrease of intensity of bands at 1200 cm^{-1} suggests breakage of hydrogen bonds between cellulose and hemicellulose. The bands at 1335 cm^{-1} and 1400 cm^{-1} indicates vibrations of the C-H bond and bending of the lignin-polysaccharide plane. The adsorption peaks at 1509 , 1464 and 1422 cm^{-1} corresponds to lignin and any changes in this region specifies lignin degradation or removal. The bands 1730 cm^{-1} and 1750 cm^{-1} represents the ester moieties in hemicellulose. Diminishing of this peak is indicative of hemicellulose removal. The adsorption peak at 2920 cm^{-1} represents C-H stretching in cellulose and the broad peaks indicates breakage of hydrogen bonds. The broadening of the bands at $3000\text{--}3500\text{ cm}^{-1}$ indicates the stretching of -OH groups.

Untreated spent coffee waste showed peaks at 897 cm^{-1} , 1035 cm^{-1} , 1200 cm^{-1} , 1400 cm^{-1} , 1750 cm^{-1} , 2920 cm^{-1} and $3000\text{ to }3500\text{ cm}^{-1}$. The FTIR spectrum corresponding to the sequentially pretreated spent coffee waste was devoid of any peaks mentioned in the above section which indicated extensive breakage of bonds between cellulose, hemicellulose and lignin. Furthermore, peaks characteristic to lignin, such as 1509 , 1464 and 1422 cm^{-1} was absent in the spectrum. Concentrated acid acetone pretreatment also showed similar characteristic in the FTIR spectrum as the sequential pretreatment. Comparisons between FTIR spectra of untreated spent coffee and

conc. acid acetone pretreatment revealed the absence of bands at 3000 to 3500 cm^{-1} indicating extensive OH stretching. The band at 1730 cm^{-1} was absent which indicated the removal of hemicellulose fractions which was in consensus with composition analysis. The peaks at 897 cm^{-1} , 1035 cm^{-1} , 1200 cm^{-1} and 1400 cm^{-1} suggesting disorderliness in the polysaccharide fractions in spent coffee waste. Peaks at 1750 cm^{-1} and 2920 cm^{-1} and the bands 3000 to 3500 cm^{-1} were prominent in organosolv pretreated spent coffee waste indicating the preservation of the hemicellulose and cellulose fractions. Dilute acid pretreatment appeared to be a very effective strategy for spent coffee waste from the differences in peak heights as opposed to the FTIR spectrum of native spent coffee waste. The FTIR of AFEX treated spent coffee waste revealed diminished peaks at 897 cm^{-1} , 1035 cm^{-1} , 1200 cm^{-1} , 1400 cm^{-1} and 1750 cm^{-1} revealing the exhaustive bond breakage on the polysaccharide and lignin fractions. AFEX and pretreatment with ferric chloride was also very effective in breaking glycosidic linkages and stretching of bonds between the polysaccharide and lignin fraction. Steam explosion also indicated strain in linkages between lignin and carbohydrates while retaining hemicellulose. According to the FTIR spectra ferric chloride pretreated spent coffee waste underwent extensive degradation in the lignin fraction and bond breakage between cellulose and hemicellulose with absence of peaks at 897 cm^{-1} , 1400 cm^{-1} and 1730 cm^{-1} and diminished peaks at 1035 cm^{-1} , 1200 cm^{-1} , 1750 cm^{-1} , 2920 cm^{-1} and bands from 3000-3500 cm^{-1} . Microwave pretreatment and plasma pretreatment conserved the hemicellulose regions as suggested by the peak at 1750 cm^{-1} .

The delignification of lignocellulose results in the aggregation of cellulose in biomass. The increase in crystallinity could affect the recalcitrance properties of lignocellulose when subjected to enzymatic hydrolysis. Hence, information on crystalline cellulose and its aggregation was critical in understanding delignification and subsequent utilisation of pretreated

biomass. The crystallinity of pretreated and native spent coffee waste was assessed by conducting X-ray diffraction. The crystallinity of SCW is contributed by the presence of cellulose. A large fraction of cellulose present in spent coffee waste is crystalline in nature. The hemicellulose component of spent coffee waste contributes to the amorphous fraction of spent coffee waste as are easily susceptible to enzymatic degradation (Ballesteros et al., 2014). Furthermore, the lignin present in spent coffee waste also contributes to the amorphous nature of spent coffee waste. The increase in crystallinity can be attributed to the effectiveness of the pretreatment. The increase in peaks at the 15° and 22° is caused by the disorderliness of the structure because of pre-treatments (Pereira et al., 2011). The spectra of different pretreated samples showed an increasing trend in the crystallinity of the biomass with respect to the effectiveness of the treatment. Absence of distinct peaks for amorphous region was observed as a general trend in the XRD spectra of all the pretreated spent coffee waste samples except for acid acetone pretreatment and alkali assisted microwave pretreatment. Sequentially pretreated spent coffee waste exhibited highest crystallinity compared to all the other pretreated spent coffee waste. Comparatively, microwave pretreated spent coffee waste exhibited lowest crystallinity among all the pretreated substrates with distinct peaks from amorphous and crystalline regions respectively. The increase in the crystalline nature of the pretreated samples can be attributed to the attrition in the amorphous components of spent coffee waste such as lignin and hemicellulose. Similar observations were reported by Binod et al. (2012) when employing short duration microwave pretreatment to improve the enzymatic hydrolysis of sugar cane bagasse. Also, Raghavi et al. (2016) reported an increase in biomass crystallinity as a result of various pretreatments.

3.5 Thermal behaviour study using differential scanning calorimetry

Thermal analytical techniques such as differential scanning calorimetry are employed to characterise the chemical and physical changes in lignocellulose. Physical transformation recorded by this DSC includes crystallisation, crystalline orientation, melting, heat capacity, glass transition etc. Tremendous effects have been made by several researchers study the lignocellulose heat interactions and thermal stability of lignocellulosic materials over the past few decades (Nguyen et al., 1981). Therefore, it was necessary to study the changes brought in on thermal behaviour of spent coffee waste by pretreatment. Differential scanning calorimetry is a technique used to determine the heat effects associated with phase transitions and chemical reactions as a function of temperature. In this method, heat flow differences in the sample and the reference (usually an empty aluminium pan) is recorded as a function of temperature. The temperature is increased at a constant rate both in the sample and reference. The heat flow is equivalent to enthalpy since the pressure is maintained constant (Ballesteros et al., 2014). Fig. 4 represents the changes in the thermal behaviour of the native and pretreated spent coffee waste between 20°C and 500°C which were obtained at a heating rate of 10°C/min under constant nitrogen atmosphere. Changes in the polymeric nature of the spent coffee waste can be observed in the thermogram. The thermogram for native spent coffee waste exhibited an exothermic event between 20°C and 102.9°C which occurs due to the vaporisation of water and crystalline nature of the sample. The enthalpy associated with this event was found to be 114.99 J/g. This was succeeded by a phase transformation with a change in the heat capacity, which is a characteristic of every polymer. The glass transition temperature was recorded at 259.2°C (Ballesteros et al., 2014). Different pretreated spent coffee waste has unique characteristics as far as their thermal behaviours were concerned. For example, microwave pretreatment exhibited an endothermic

event and an exothermic event followed by a crystalline peak, which was specific to this type of sample. The thermograms of pretreated samples such as steam explosion, ferric chloride assisted treatment, plasma, organosolv, and AFEX were all similar to that of the native spent coffee waste except for the variations in enthalpy. However, thermograms of conc. phosphoric acid acetone treated biomass and spent coffee waste that underwent sequential pretreatment were similar to each other while being unique from the rest of the pretreatments. The thermograms of these two pretreatments reported glass transition followed by a crystallisation peak, melting peak and finally an ending transient. Changes in the lignin and hemicellulose content can result in shifting of peak formation along the temperature variations of the calorimetric process. Tsujiyama et al. (2001) in their study using DSC to analyse the changes brought about in wood by brown rot fungi degradation observed shifts in the main peak from a lower temperature region to a higher temperature region with respect to the degradation of lignin and hemicellulose. The prominence of hemicellulose due to the decay of lignin can result in the emergence of a peak at lower temperature ranges. Comparing the DSC thermograms of native and sequential pretreated spent coffee waste there was shift in the main peak from 260° to 282° indicating loss of lignin and cellulose content. Furthermore, pretreated spent coffee waste with higher hemicellulose content was observed to have their main peaks shifted to lower temperature regions (e.g. organosolv and microwave pretreatment).

4 Conclusion

A sequential strategy to combine the effects of two pretreatments was found to be highly efficient in increasing the reducing sugar yield. A 1.7-fold increase in reducing sugar yield was obtained after enzymatic hydrolysis of sequential pretreated spent coffee waste as compared to native spent coffee waste. FTIR analysis revealed breakage of β -glycosidic bond between

cellulose and hemicellulose as well as lignin removal. XRD spectra revealed an increasing trend in crystallinity, due to the removal of amorphous structures in spent coffee waste. SEM images of the biomass that was subjected to sequential pretreatment exhibited a crumbled structure as opposed to native spent coffee waste.

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References

1. Adney, B., Baker, J. 1996. Measurement of cellulase activities. Laboratory Analytical Procedure, 6, 1996.
2. Ballesteros, L.F., Cerqueira, M.A., Teixeira, J.A., Mussatto, S.I. 2015. Characterization of polysaccharides extracted from spent coffee grounds by alkali pretreatment. Carbohydr. Polym. 127, 347-354.
3. Ballesteros, L.F., Teixeira, J.A., Mussatto, S.I. 2014. Chemical, Functional, and Structural Properties of Spent Coffee Grounds and Coffee Silverskin. Food Bioprocess Technol. 7, 3493-3503.
4. Binod, P., Satyanagalakshmi, K., Sindhu, R., Janu, K.U., Sukumaran, R.K., Pandey, A. 2012. Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse. Renew. Energ. 37, 109-116.
5. Campos-Vega, R., Loarca-Piña, G., Vergara-Castañeda, H.A., Oomah, B.D. 2015. Spent coffee grounds: A review on current research and future prospects. Trends Food Sci. Technol. 45, 24-36.

6. Chen, L., Chen, R., Fu, S. 2015. FeCl₃ pretreatment of three lignocellulosic biomass for ethanol production. *ACS Sustain. Chem. Eng.* 3, 1794-1800.
7. Chiyanzy, I., Brienzo, M., García-Aparicio, M., Agudelo, R., Görgens, J. 2015. Spent coffee ground mass solubilisation by steam explosion and enzymatic hydrolysis. *J. Chem. Technol. Biotechnol.* 90, 449-458.
8. Go, A.W., Conag, A.T., Cuizon, D.E.S. 2016. Recovery of Sugars and Lipids from Spent Coffee Grounds: A New Approach. *Waste Biomass Valori.* 7, 1047-1053.
9. Huang, Y., Wei, X., Zhou, S., Liu, M., Tu, Y., Li, A., Chen, P., Wang, Y., Zhang, X., Tai, H., Peng, L., Xia, T. 2015. Steam explosion distinctively enhances biomass enzymatic saccharification of cotton stalks by largely reducing cellulose polymerization degree in *G. barbadense* and *G. hirsutum*. *Bioresour. Technol.* 181, 224-230.
10. Jaiswal, A.K., Gupta, S., Abu Ghannam, N. 2012. Optimisation of lactic acid fermentation of York cabbage for the development of potential probiotic products. *Int. J. Food Sci. Technol.* 47, 1605-1612.
11. Jooste, T., García-Aparicio, M., Brienzo, M., Van Zyl, W., Görgens, J. 2013. Enzymatic hydrolysis of spent coffee ground. *Appl. Biochem. Biotechnol.* 169, 2248-2262.
12. Kwon, E.E., Yi, H., Jeon, Y.J. 2013. Sequential co-production of biodiesel and bioethanol with spent coffee grounds. *Bioresour. Technol.* 136, 475-480.
13. Li, H., Kim, N.-J., Jiang, M., Kang, J.W., Chang, H.N. 2009. Simultaneous saccharification and fermentation of lignocellulosic residues pretreated with phosphoric acid–acetone for bioethanol production. *Bioresour. Technol.* 100, 3245-3251.
14. Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 31, 426-428.

15. Murthy, P.S., Naidu, M.M. 2012. Production and Application of Xylanase from *Penicillium* sp. Utilizing Coffee By-products. Food Bioprocess Technol. 5, 657-664.
16. Mussatto, S.I., Carneiro, L.M., Silva, J.P., Roberto, I.C., Teixeira, J.A. 2011. A study on chemical constituents and sugars extraction from spent coffee grounds. Carbohydr. Polym. 83, 368-374.
17. Nguyen, T., Zavarin, E., barrall, E.M. 1981. Thermal Analysis of Lignocellulosic Materials. J. Macromolecular Sci. Part C, 20, 1-65.
18. Ostovareh, S., Karimi, K., Zamani, A. 2015. Efficient conversion of sweet sorghum stalks to biogas and ethanol using organosolv pretreatment. Indus. Crops Products. 66, 170-177.
19. Pereira, P.H.F., Voorwald, H.C.J., Cioffi, M.O.H., Mullinari, D.R., Da Luz, S.M., Da Silva, M.L.C.P. 2011. Sugarcane bagasse pulping and bleaching: Thermal and chemical characterization. BioResources, 6, 2471-2482.
20. Raghavi, S., Sindhu, R., Binod, P., Gnansounou, E., Pandey, A. 2016. Development of a novel sequential pretreatment strategy for the production of bioethanol from sugarcane trash. Bioresour. Technol. 199, 202-210.
21. Ravindran, R., Jaiswal, A.K. 2016a. Exploitation of food industry waste for high-value products. Trends in Biotechnol. 34, 58-69.
22. Ravindran, R., Jaiswal, A.K. 2016b. A comprehensive review on pre-treatment strategy for lignocellulosic food industry waste: challenges and opportunities. Bioresour. Technol. 199, 92-102.
23. Ravindran, R., Jaiswal, S., Abu-Ghannam, N., Jaiswal, A.K. 2017. Evaluation of ultrasound assisted potassium permanganate pre-treatment of spent coffee waste. Bioresour. Technol. 224, 680-687.

24. Rickard, P., Laughlin, T. 1980. Detection and assay of xylanolytic enzymes in a *Cellulomonas* isolate. *Biotechnol. Lett.* 2, 363-368.
25. Rocha, M.V.P., de Matos, L.J.B.L., Lima, L.P.d., Figueiredo, P.M.d.S., Lucena, I.L., Fernandes, F.A.N., Gonçalves, L.R.B. 2014. Ultrasound-assisted production of biodiesel and ethanol from spent coffee grounds. *Bioresour. Technol.* 167, 343-348.
26. Scully, D.S., Jaiswal, A.K., Abu-Ghannam, N. 2016. An Investigation into spent coffee waste as a renewable source of bioactive compounds and industrially important sugars. *Bioengineering*, 3, 33 (DOI:10.3390/bioengineering3040033).
27. Shao, Q., Cheng, C., Ong, R.G., Zhu, L., Zhao, C. 2013. Hydrogen peroxide presoaking of bamboo prior to AFEX pretreatment and impact on enzymatic conversion to fermentable sugars. *Bioresour. Technol.* 142, 26-31.
28. Siripong, P., Duangporn, P., Takata, E., Tsutsumi, Y. 2016. Phosphoric acid pretreatment of *Achyranthes aspera* and *Sida acuta* weed biomass to improve enzymatic hydrolysis. *Bioresour. Technol.* 203, 303-308.
29. Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D. 2005. Determination of extractives in biomass. *Laboratory Analytical Procedure (LAP)*, 1617.
30. Tsujiyama, Sho-ichi. 2001. Differential scanning calorimetric analysis of the lignin-carbohydrate complex degraded by wood-rotting fungi. *J. Wood Sci.* 47, 497-501.
31. Uppugundla, N., da Costa Sousa, L., Chundawat, S.P., Yu, X., Simmons, B., Singh, S., Gao, X., Kumar, R., Wyman, C.E., Dale, B.E., Balan, V. 2014. A comparative study of ethanol production using dilute acid, ionic liquid and AFEX™ pretreated corn stover. *Biotechnol. Biofuels*, 7, 1-14.

32. Utomo, H.D., Hunter, K. 2006. Adsorption of divalent copper, zinc, cadmium and lead ions from aqueous solution by waste tea and coffee adsorbents. *Environment. Technol.* 27, 25-32.
33. Zhao, X., Cheng, K., Liu, D. 2009. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. *Appl. Microbiol. Biotechnol.* 82, 815.
34. Zheng, Y., Lee, C., Yu, C., Cheng, Y.-S., Zhang, R., Jenkins, B.M., VanderGheynst, J.S. 2013. Dilute acid pretreatment and fermentation of sugar beet pulp to ethanol. *Appl. Energ.* 105, 1-7.

Highlights

- Spent coffee waste was subjected to eight different pretreatments.
- XRD, FTIR, and DSC profiles of the pretreated substrates were analysed.
- A sequential pretreatment strategy was proposed by combining the best pretreatments.
- Sequential pretreatment yielded 1.7-fold higher reducing sugar content.
- Galactose was found to be the predominant sugar in SCW.

Fig. 1. Proposed sequential pretreatment

Fig. 2a: Dilute acid hydrolysis (effect of acid concentration and time on reducing sugar release)

Fig. 2b: Pressure plasma pretreatments (effect of voltage and time on reducing sugar release)

Fig. 3. Total reducing sugar released after enzymatic hydrolysis of pretreated spent coffee waste

Fig. 4. DSC thermogram of untreated and pretreated spent coffee waste

Table 1: Component analysis of SCW after different pretreatments

Pretreatment	Cellulose	Galactan	Arabinan	Mannan	AIL	ASL
Plasma	12.65±0.1 ^a	20.08±0.9 ^a	1.89±0.4 ^a	16.88±0.8 ^a	16.12±0.1 ^a	3.65±0.1 ^a
Ferric Chloride	10.74±0.9 ^b	18.76±0.2 ^b	2.24±1.2 ^b	24.51±1.3 ^b	12.32±0.45 ^b	3.11±0.5 ^{bc}
Organosolv	7.04±3.1 ^c	30.12±0.4 ^c	3.02±0.3 ^c	31.54±0.5 ^c	14.45±0.12 ^c	2.89±0.6 ^b
Microwave-alkali	11.02±2.7 ^b	25.67±0.5 ^d	2.24±0.6 ^b	14.78±0.7 ^d	21.20±0.4 ^d	2.40±3.1 ^d
Dil. Acid Hydrolysis	15.37±0.2 ^d	3.84±0.5 ^e	0.476±1.1 ^d	5.88±2.4 ^e	25.67±0.3 ^e	1.17±2.1 ^e
AFEX	8.70±1.2 ^c	5.21±0.8 ^f	0.62±0.2 ^d	9.41±0.1 ^f	12.20±3.4 ^b	3.97±0.1 ^f
Steam Explosion	6.20±0.4 ^c	12.40±1.2 ^g	3.10±0.7 ^c	20.2±0.3 ^g	24.50±0.9 ^g	3.17±0.2 ^d
Conc. Phosphoric Acid acetone	18.14±0.5 ^f	2.40±0.3 ^h	0.42±0.6 ^d	4.75±0.1 ^h	21.43±0.4 ^h	1.06±0.4 ^e
Sequential Pretreatment	20.01±0.2 ^g	2.10±0.3 ^h	0.39±1.2 ^d	4.05±0.3 ⁱ	10.30±0.2 ⁱ	1.04±0.9 ^e
Control	8.60±0.1 ^h	13.7±0.3 ⁱ	1.70±0.2 ^a	21.10±0.5 ^j	27.12±0.4 ^j	3.89±0.3 ^{af}

All results are presented as g/100g of dry SCW. Means not sharing the same letter are significantly different (LSD) at $P < 0.05$ probability level for respective lignocellulosic components.